Remarks

This amendment is filed in response to the Communication from the Patent Office mailed on December 22, 2004. This amendment is substantially the same as the Amendment filed with the Response to Office Action mailed on September 20, 2004, with formatting changed to comply with 37 CFR 1.121(c)(3). Specifically, claims 47-54, identified as "withdrawn", now include the entire text of the claim.

Claims 37, 39, 41-46 and 55 were previously pending in this application. By this amendment, claim 37 has been amended. As a result claims 37, 39, 41-46 and 55 are pending for examination with claim 37 being an independent claim. Support for the amendment to the claims is supported by the specification and claims as filed. No new matter has been added.

Objection under 37 CFR 1.75(c)

Claim 43 is objected to under 37 CFR 1.75(c) as being in improper dependent form for failing to further limit the subject matter of the previous claim. The Examiner argues that the requirement in claim 43 for the antigen and the non-toxic double mutant form of pertussis toxin to be "administered at the same time" does not limit the requirement in claim 37 that the two are "co-administered". Applicant respectfully traverses.

The requirement of claim 43 that the two components are "administered at the same time" does further limit the requirement of claim 37 that the two components are "co-administered". The requirement for "co-administration" allows for the two components to be administered one after the other, i.e. with a short time lag between administration of the first component and administration of the second component. In contrast, the requirement for the two components to be "administered at the same time" requires that they are administered simultaneously. The specification makes clear in the last complete paragraph on page 9 that the two components may be administered separately at slightly different times.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the objection under 37 CFR 1.75(c).

Rejection under 35 USC 112, first paragraph

Claims 37, 39, 41-46 and 55 are rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement.

Applicant has cancelled the limitation in claim 37 that the antigen is not pertussis toxin. This renders the rejection moot.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 USC 112, first paragraph.

Rejection under 35 USC 103(a)

Wilson et al. in view of Nencioni et al.

Claims 37, 39, 41, 43, 44, 46 and 55 are rejected under 35 USC 103(a) as being unpatentable over Wilson et al. (Vaccine, 11(2):113-118, 1993) in view of Nencioni et al. (Acta Med. Rom. 29:78-83, 1991).

The claimed invention is concerned with a method of using a non-toxic double mutant of pertussis toxin as a mucosal adjuvant, i.e. as a substance that stimulates or enhances a protective immune response to an antigen that is co-administered to a mucosal surface with the mutant pertussis toxin. The pertussis toxin has mutations at positions 9 and 129 of the S1 subunit which render it non-toxic by inactivating the ADP-ribosylating enzymatic activity of the native toxin.

It was not obvious that the mutant pertussis toxin as recited in the claims would be an effective mucosal adjuvant. The perception in the art was that the adjuvant activity of pertussis toxin was likely to be inseparable from its enzymatic activity, and it was therefore expected that inactivating the enzymatic activity of the toxin would also inactivate its adjuvant activity. However, the Inventor actually found the opposite to be the case; he found that, if anything, the mutant pertussis toxin as recited in the claims is a more effective mucosal adjuvant than the wild-type toxin. That was totally unexpected.

However, the Examiner argues that Wilson et al. teach the adjuvant activity appears independent of the enzymatic activity of the S1 subunit and as such one skilled in the art would have reasonably expected at the time the invention was made that the modified S1 subunit would also have the adjuvant function of the native S1 toxin of the art. Applicant respectfully submits

that the Examiner's argument is unfounded and is based on an incomplete understanding of Wilson et al.

A Declaration from Inventor Mark Roberts which explains what can in fact be concluded from Wilson et al. is provided herewith. (The presently submitted Declaration is unsigned, but a signed version will be forwarded to the Examiner shortly.) Dr. Roberts explains that pertussis toxin produces a myriad of biological effects by catalysing the ADP-ribosylation of certain G proteins. Wilson et al. tried to examine just one of these effects, namely the elevation of cAMP levels. Thus, even if Wilson et al. did show that elevation of cAMP has no effect on adjuvant activity (which they did not for reasons explained below), this would not allow any conclusion to be drawn that the adjuvant activity of pertussis toxin is independent of its enzymatic activity. The most that could be concluded is that the adjuvant activity of pertussis toxin is probably mediated through an effect of its enzymatic activity different from its effect on cAMP levels. This is recognised in the last paragraph of the Discussion section of Wilson et al., where it is stated that:

> "Although this experiment is a rather blunt probe of immune regulation we consider that CT and PT may act by an alternative mechanism, such as via a common G protein-mediated effect not involving an enhancement of adenylate cyclase activity."

Thus, even the authors of Wilson et al. recognise that, even if their results are taken as face value, the effect of pertussis toxin on immune regulation is likely to derive from a G protein-mediated effect (i.e. an enzyme-mediated effect) of the toxin not involving elevation of cAMP. In other words, the authors recognise that the effect of pertussis toxin on immune regulation is likely to derive from one of the myriad of non-cAMP related effects resulting from the enzymatic activity of pertussis toxin.

Dr. Roberts gives a second reason why it cannot be concluded from Wilson et al. that the adjuvant activity of pertussis toxin is independent of the enzymatic activity of the toxin. In particular, Dr. Roberts explains in paragraph 6 of his Declaration that:

> "Wilson et al. did not in fact show that they had produced any effect on cAMP levels. The relevant experiment described in Wilson et al. involves feeding forskolin to mice. Forskolin is known to raise cAMP levels in cultured cells *in*

vitro. However, Wilson et al. does not show that feeding forskolin to mice produces elevated cAMP levels or has any other relevant effect. All that Wilson et al. showed was that the mice got sick. They did not show that feeding forskolin had any relevant effect on the immune function of the mice."

In summary, Dr. Roberts finds that:

"it cannot be concluded from Wilson et al. that the adjuvant activity of pertussis toxin is independent of the enzymatic activity of the toxin."

(Paragraph 3 of Dr. Roberts' Declaration).

There was in fact clear evidence in the art that the adjuvant activity of pertussis toxin is dependent upon its enzymatic activity. For example, Black et al. (1988) Science 240, 656-659 described an experiment in which it was shown that heat-killed whole cells of *B. pertussis* strains which had a four amino acid insertion in the S1 subunit of pertussis toxin resulting in a 90% drop in enzymatic activity did not enhance the serum antibody response to ovalbumin, whereas killed cells prepared from strains with wild-type pertussis toxin genes did. Black et al. concluded that "this mutant strain failed to function as an adjuvant".

The Examiner is further directed to Holmgren et al. (September 1993) Vaccine 11(12), 1179-1184, which is already of record in the parent application. Holmgren et al. is a review of the use of cholera toxin (CT) and the B subunit of cholera toxin as an oral-mucosal adjuvant and antigen vector systems. Holmgren et al. also discusses the heat-labile toxin (LT) from *E. coli*. Pertussis toxin is closely related to both cholera toxin and heat-labile toxin in the sense that all three are bacterial toxins, all three have an AB_5 subunit structure (A = active subunit, B = binding subunit), the A subunits of all three have ADP-ribosylating enzymatic activity and all three have adjuvant activity. Thus, persons ordinarily skilled in the art believed that what was true of one of the toxins was generally also likely to be true of the other two.

Holmgren et al. contains a section entitled "Can adjuvanticity be separated from enterotoxicity?" (see pages 1182-1183). The experiments described in this section of Holmgren et al. clearly suggest that the answer to this question is "no".

See, for example, the sub-section bridging pages 1182 and 1183 of Holmgren et al. entitled "Single amino acid substitution in the A subunit associated with concomitantly loss of enterotoxic and adjuvant activities". This sub-section describes experiments which involved use

of a mutant LT with a single amino acid substitution. The mutant LT was known to be completely devoid of toxicity. Holmgren et al. showed that the mutant is also completely devoid of adjuvant activity; they showed that the mutant "fails completely to stimulate an immune response to KLH" (Holmgren et al., page 1183, left column, lines 18-19).

Holmgren et al. described the mutant LT with a single amino acid substitution as "an excellent tool" to address the question of whether enterotoxicity and adjuvanticity are separable. The results clearly suggest that they are not separable.

Thus, the Inventor's results as shown in e.g. the Examples of the patent application are the opposite of what would have been expected. The mutations as positions 9 and 129 recited in the claims are designed to destroy the enzymatic and toxic activity of the toxin. The Inventor found that the mutations have, if anything, the opposite effect on the ability of the toxin to act as an adjuvant. That was not obvious.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 USC 103(a).

Wilson et al., Nencioni et al., Capiau et al. and Tamura et al.

Claim 42 is rejected under 35 USC 103(a) as being unpatentable over Wilson et al. and Nencioni et al. and in further view of Capiau et al. (EP 352250, published 01.24.90) and Tamura et al. (US Patent 5,182,109).

Claim 42 is dependent upon claim 37. Applicant submits that claim 42 is patentable for the same reasons as given above for claim 37.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 USC 103(a).

Wilson et al., Nencioni et al. and Halpern et al.

Claim 45 is rejected under 35 USC 103(a) as being unpatentable over Wilson et al. and Nencioni et al. and in further view of Halpern et al. (Infection and Immunity, 58(4):1104-1009, 1990).

Claim 45 is dependent upon claim 37. Applicant submits that claim 45 is patentable for the same reasons as given above for claim 37.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 USC 103(a).

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,

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